Environ. Sci. Technol. **2008,** *42,* 5521–5526

Transport of Testosterone and Estrogen from Dairy-Farm Waste Lagoons to Groundwater

SHAI ARNON,*,† OFER DAHAN,† SARA ELHANANY,‡ KEREN COHEN,§ IRENA PANKRATOV,‡ AMIT GROSS,† ZEEV RONEN,† SHAHAR BARAM,† AND LAURENCE S. SHORE§

Department of Environmental Hydrology and Microbiology, Zuckerberg Institute for Water Research, J. Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede Boqer 84990, Israel, Israel Water Authority, 14 Hamasger Street, Tel-Aviv, 61203, Israel, and Kimron Veterinary Institute, P.O. Box 12, Bet Dagan, 50250, Israel

Received March 19, 2008. Revised manuscript received May 12, 2008. Accepted May 19, 2008.

Although concentrated animal feeding operations constantly generate physiologically active steroidal hormones, little is known of their environmental fate. Estrogen and testosterone concentrations in groundwater and their distribution in sediments below a dairy-farm wastewater lagoon were therefore determined and compared to a reference site located upgradient of the farm. Forward simulations of flow as well as estrogen and testosterone transport were conducted based on data from the sediment profile obtained during drilling of a monitoring well below the dairy-farm waste lagoon. Testosterone and estrogen were detected in sediments to depths of 45 and 32 m, respectively. Groundwater samples were directly impacted by the dairy farm, as evidenced by elevated concentrations of nitrate, chloride, testosterone, and estrogen as compared to the reference site. Modeling potential transport of hormones in the vadose zone via advection, dispersion, and sorption could not explain the depths at which estrogen and testosterone were found, suggesting that other transport mechanisms influence hormone transport under field conditions. These mechanisms may involve interactions between hormones and manure as well as preferential flow paths, leading to enhanced transport rates. These types of interactions should be further investigated to understand the processes regulating hormone transport in the subsurface environment and parametrized to forecast long-term fate and transport of steroidal hormones.

Introduction

Wastewater lagoons are standard practice for the storage and treatment of manure from concentrated animal feeding operations (CAFOs). Many states require that the soil lining at the bottom of the wastewater lagoons have a saturated hydraulic conductivity of $<10^{-6}$ cm/s (*1*), making soil lining an attractive, cost-effective solution for waste storage as compared to plastic lining. However, soil lining has repeatedly failed to reduce deep infiltration of water and contaminant leaching into the groundwater, resulting in extensive groundwater contamination with chloride, nitrate, and, in some cases, bacterial pathogens as well (*2–6*). Only a small fraction of the studies examining the impact of CAFOs on groundwater quality has included hormones in their measurements, despite the fact that a link between animal farming and surface water contamination with hormones was shown over 25 years ago (*7*).

Steroid hormones are phylogenetically old chemical substances that have been found to have physiological roles in all phyla higher than, and including, cnidarians and molluscans (*8*). The primary steroid hormones are estrone, estradiol, progesterone, testosterone, and cortisol. Excess amounts of hormones in the environment are hazardous because they have the potential to cause adverse health effects in humans and wildlife by interfering with the normal activity of hormones involved in growth control, metabolism, and other body functions (*9*). The steroids of major concern are estrone and 17β-estradiol because they exert their physiological effects at lower concentrations than other steroids and can be found in the environment at concentrations above their lowest observable effect level (LOEL) for fish and plants (10 ng/L) (*10, 11*). The common concentrations of progesterone and testosterone in the environment are at least 1 order of magnitude below their LOEL. Most hormones are lipophilic in nature and therefore poorly soluble in water (log K_{ow} between 2.6 and 4 *11, 12*). Therefore, it is expected that hormones will be adsorbed to sediment particles and organic carbon and will have a limited ability to move into the subsurface environment, as shown by several sorption and column studies (*13–16*). Moreover, as organic molecules, hormones are likely susceptible to microbial degradation. Nevertheless, in recent years, there has been a rise in cases in which hormones were found at depths of more than several meters and in groundwater (*17–20*). For example, Swartz et al. (*18*) measured estrone and 17β-estradiol, which had leached from wastewater tanks through glacial deposits of sand and gravel, in groundwater at depths of 3−6 m and concentrations of up to 120 and 45 ng/L, respectively. Wicks et al. (*17*) measured 17β-estradiol in a karstic aquifer at concentrations of up to 80 ng/L. However, in those studies, the mechanism of transport through the unsaturated zone was not discussed.

In this study, we measured for the first time the deep vertical distribution of estrogen and testosterone in unsaturated sediments below a CAFO waste lagoon and conducted solute transport simulations to test the hypothesis that solute advection, dispersion, and sorption cannot fully explain the distribution of hormones in an unsaturated zone. Understanding the behavior and transport of steroidal hormones in the subsurface can improve our ability to protect our groundwater resources and may provide tools for the development of remediation strategies.

Materials and Methods

Study Site. A dairy farm with ~60 dairy cows and 30 calves was chosen for the study of testosterone and estrogen transport from a waste lagoon to groundwater through the vadose zone. The dairy farm is located above the southern section of the Israeli Coastal Aquifer. The facility uses a 150 m^2 single-stage earthen unlined waste lagoon with an average depth of 0.5 m, which is common manure-management practice in the area. Excess wastewater overflows directly into a dry creek, while no specific maintenance procedures, such as drainage or solid removal, are used at the site.

* Corresponding author phone: 972-8-6563510; fax: 972-8-6596909; e-mail: sarnon@bgu.ac.il.

† Ben-Gurion University of the Negev.

‡ Israel Water Authority.

§ Kimron Veterinary Institute.

10.1021/es800784m CCC: $40.75
Published on Web 06/28/2008

Two 6 in. boreholes were drilled for the purposes of sediment sampling and construction of groundwater-monitoring wells. One borehole was drilled directly under the waste lagoon (RFW1), after a small portion of the lagoon was dried. A second borehole (RFW2) was drilled ~1 km east of the dairy farm (upgradient), in a typical agricultural field used for growing barley. The boreholes were entirely cased with PVC pipes and perforated from ~1 m above the groundwater surface to the bottom of the borehole, which was ~7 m below groundwater level (groundwater levels were 47 and 42 m below ground surface for RFW1 and RFW2, respectively).

Sediment and Groundwater Sampling. A dry-drilling method with a bucket auger was used for sediment sampling from the entire vadose zone cross-section. The auger was cleaned between samples with pressurized water and a propane flame to prevent cross-contamination. Sediment samples were collected during the drilling at uneven intervals, with denser sampling at the top of the profile (20 samples under the waste lagoon and 11 samples under the reference site: see SI1, Supporting Information, for more details). Sediments were stored in plastic bags and kept on ice until they reached the laboratory (<24 h) and then were stored at 4 °C for further analysis. Groundwater was sampled using a submersible pump (model MP1, Grundfos, Denmark) after purging three well-volumes. Samples for major ions were stored in PVC bottles, while samples for hormones were stored in glass bottles equipped with Teflon caps and kept on ice until they reached the laboratory (<24 h). In addition, groundwater samples for major-ion analysis were filtered through a 45 μm filter and acidified to pH 2 until analysis (<2 weeks).

Extraction Methods. Sediments were extracted for major-ion analysis by taking 5 g of sediment in 15 mL of distilled water. The sediment slurries were shaken at 150 rpm for 15 min, before the supernatant was separated from the sediment by centrifugation at 3000 rpm for 20 min, filtered through a 45 μm filter, and acidified to pH 2 until analysis (<2 weeks).

Sediments were extracted for testosterone and estrogen by placing 5 g of dried sediment, 5 mL of distilled water, and 10 mL of ethyl acetate (EA) in capped vials. The sediment slurries were shaken at 150 rpm for 24 h. The organic solvent was removed using a glass pipet, and the aqueous layer was re-extracted with 10 mL of EA. The combined supernatants were placed in a glass vial and evaporated to dryness under a hood. The residue was then redissolved in 2 mL of methanol and kept at 4 °C until analysis. Waste lagoon slurries, which contained ~30% solid material, were extracted by the same procedure. Dried dairy cattle manure samples were extracted with methanol as previously described for chicken manure (*21*).

Groundwater samples were extracted for hormone analysis by mixing 500 mL of the sample with 200 mL of 0.1 N sodium acetate buffer. The mixture was passed through a C_{18} solid-phase column, eluted with methanol, evaporated to dryness, and redissolved in sodium acetate buffer for analysis.

Chemical Analysis. Nitrate and chloride were analyzed using an ion chromatograph (Dionex). Testosterone and estrogen were measured by radioimmunoassay following Shore and Shemesh (*22*). The detection limit for testosterone and estrogen was 0.3 ng/L, with a hormone recovery of 90% if samples were above 1.0 ng/L. However, below 0.5 ng/L, recoveries were <50% as determined by LC/MS (*23*). The antibody for testosterone recognized both testosterone and dehydrotestosterone (50%). The antibody for estrogen recognized both estrone and estradiol (50%) and exhibited only negligible cross-reactions with other steroids.

Modeling Flow and Solute Transport under Dairy-Farm Waste Lagoon. The flow, transport, and distribution of water content and hormones in the vadose zone below a dairy-farm waste lagoon was modeled using the computer program HYDRUS-1D version 3 (*24*). HYDRUS-1D solves the Richards equation numerically for saturated/unsaturated water flow and convection/dispersion-type equations for solute transport.

Forward modeling over 40 years of operation was used in all of our simulations since the waste-management practices in the farm have not changed considerably over the years (farm owner, personal communication). The model considers the sediment properties (mineral types and hydraulic conductivity), a continuous infiltration of water under constant hydraulic conditions, a continuous variable input of testosterone and estrogen, and sorption of hormones onto mineral and organic matter. The model consists of the entire vadose zone divided into three homogeneous layers (6 m of clay, followed by a 2 m transition layer of sandy loam, and 37 m of calcareous sand), based on sediment analysis from drilling under the waste lagoon (SI1, Supporting Information). The top boundary was covered by the waste-water lagoon at a constant head pressure of 0.5 m (controlled by an overflow system), while the bottom boundary (45 m) was the groundwater level with zero pressure. Initial water content was taken as the typical value for similar sediments as measured on samples from a nearby agricultural field. Particle-size distribution and mineral composition were measured on three representative samples from RFW1. These values served for the general classification of sediment type and their hydraulic characterization since not all of the variables were measured directly. Simulations were conducted under a wide range of properties for these sediment types under various disparate scenarios to show the potential migration of solutes under the dairy farm. Saturated hydraulic conductivity of the upper clay layer was measured in situ (in the waste lagoon) using a standard falling head test (*25*), while the saturated hydraulic conductivity of the other sediment types was calculated based on particle-size distribution using the neural network prediction embedded in HYDRUS (Rosseta lite, v 1.1).

Adsorption of hormones was considered to occur under equilibrium conditions since we estimated that the slow water velocity in the clay would prevent mass-transfer constraints. A wide range of adsorption coefficients was used based on the literature (*15, 16, 26, 27*), while both linear and Freundlich models were used in our simulations. We used a continuous variable concentration at the boundary with repeated half-year cycles of 750 and 1500 ng/L for estrogen and 750 and 900 ng/L for testosterone. These concentrations reflect our measured estrogen and testosterone concentrations in the waste lagoon [1154 ± 540 ng/L for estrogen ($n = 3$) and 840 ± 75 ng/L for testosterone ($n = 3$)]. On the basis of hormone concentrations in the dairy cattle manure (25 000 ng/kg testosterone and 300 000 ng/kg estrogen) for 50 lactating cows, if all of the hormones were dissolved in the 70 L of slurry produced each day, the concentrations would be 2500 and 33 000 ng/L for testosterone and estrogens, respectively (*22*). The lower observed amounts of dissolved hormones in the slurry are probably the result of limited solubility of steroid hormones and their partial degradation.

Results and Discussion

Sediment Properties. Table 1 displays the properties of the three types of sediment that were found under the waste lagoon. The upper section of the profile (0−6 m) is characterized by a high content of clay minerals and organic matter as compared to the sediments below a depth of 6 m. Below a depth of 8 m, the sediments were primarily sand with a high calcareous content and low organic matter, while between 6 and 8 m, there is a transition layer of sandy loam.

Distribution of Testosterone and Estrogen under Dairy-Farm Waste Lagoon and Agricultural Field. Testosterone

TABLE 1. Selected Properties of Sediments at the Study Site

depth (m)	sand (%)	silt (%)	clay (%)	organic matter content (%)	mineral composition (%)				
					illite-smectite and kaolinite	quartz	calcite	K-feldspar	plagioclase
1.35	16	33	51	2.24	45	35	10	5	5
6.35	53	19	28	1.13	15	85			
25	79	13	8	0.38	10	35	50		5−10

and estrogen were found along the deep sediment profile under the waste lagoon and were virtually absent in the sediment profile in the agricultural field (Figure 1). The one exception at the top section of the sediment profile in the agricultural field can be attributed to the natural activity of animals, such as dogs, snails, etc. since manure was not used to fertilize the soil. Under the waste lagoon, testosterone was detected all through the sediment profile, down to the groundwater surface, while estrogen was detectable to a depth of 32 m. The presence of testosterone at depths of >45 m below the waste lagoon (and its absence under the agricultural field) suggests that leachates from the dairy farm traverse the entire vadose zone and reach the groundwater. This is further supported by the high chloride and nitrate concentrations found in the groundwater below the waste lagoon as compared to that below the agricultural field (Figure 2). Neither testosterone nor estrogen was detected in the groundwater below the agricultural field, but they were measured at relatively low concentrations (below their LOEL) under the dairy farm (Figure 2).

The results presented in Figures 1 and 2 are surprising because batch and column experiments have repeatedly suggested that hormones are expected to sorb strongly to soil and sediments such that their distribution would be limited to the top layers (*14–16*). To our best knowledge, neither testosterone nor estrogen has ever been detected at such depths. However, some recent measurements in shallow groundwater (<5 m from the surface) have shown that hormones can reach the groundwater (*17, 18, 28*), suggesting that other mechanisms may be enhancing their mobilization. Recently, Stumpe and Marschner (*27*) demonstrated that the presence of manure reduces the distribution coefficient, K_d, of estrogen and increases the distribution coefficient of testosterone. It also was hypothesized by Hanselman et al. (*29*) that the presence of surfactants and colloids might increase the mobility of estrogens in the environment.

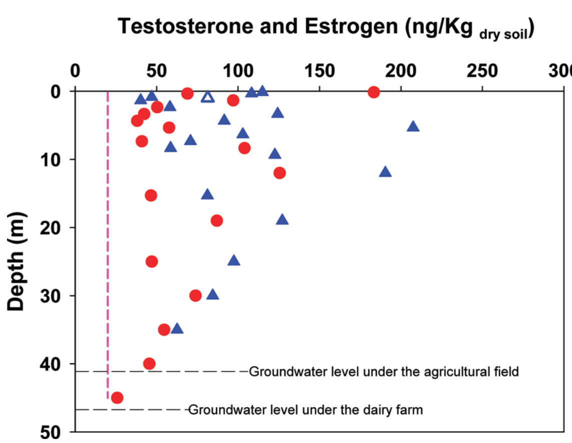

FIGURE 1. Concentrations of testosterone (●) and estrogen (▲) in the sediment profile under the dairy-farm waste lagoon (solid symbols) and agricultural field (open symbols). Detection limit for the steroidal hormones is marked with a dashed vertical line; groundwater level is marked with a horizontal dashed line.

Nevertheless, direct evidence of enhanced hormone transport in the unsaturated zone has still not been reported in the literature, and solute transport is still widely discussed as the governing mechanism of transport. To evaluate the potential extent of hormone penetration as solutes under the clay-lined waste lagoon, we conducted a set of forward simulations using HYDRUS-1D and compared the results to the hormone distribution in the sediment profile (Figure 1).

Forward Modeling of Testosterone and Estrogen Transport. Modeling of testosterone and estrogen transport requires initial calibration of the flow model. Figure 3 displays the water-content distribution as measured gravimetrically on sediment samples, as well as eight other modeling scenarios. In all eight simulations, the water content of the clay was similar, the water content of the sandy loam varied between 20 and 23%, and the water content of the sand varied between 5 and 12%. The different scenarios were selected following a sensitivity analysis that identified the most influential parameters (Table 2 and SI2, Supporting Information) and represent the range of conditions that potentially existed in the vadose zone. The most critical parameter controlling the water content and flux in the entire vadose zone was the hydraulic conductivity of the clay layer, $K_{s\text{-clay}}$. Other significant parameters were $Q_{r\text{-sand}}$, n_{sand}, and $K_{s\text{-sand}}$, where Q_r is the residual water content and n is a fitting parameter in the soil−water-retention function (SI2, Supporting Information). In addition, the observed water content in the sandy layers could only be simulated with $K_{s\text{-clay}}$ values lower than 0.17 cm/day, which is in agreement with the measured hydraulic conductivity of the bottom of the pond as was found by using in situ falling head permeameters (0.02 ± 0.01 cm/day, $n = 9$). $K_{s\text{-clay}}$ values above 0.17 cm/day yielded a high water content in the sand (>13%), far above the measured values, and they were therefore excluded from further analyses. A decreasing $K_{s\text{-clay}}$ value resulted in a decrease in the overall flow rates through the vadose zone, while the other significant parameters affected only the distribution of water content. For example, under a high $K_{s\text{-clay}}$ value (0.17 cm/day), the calculated average pore water velocities were 1.7 and 6.9 m/year in the clay and sandy layers, respectively, while under low $K_{s\text{-clay}}$ values (0.08 cm/day), the calculated average pore water velocities were, respectively, 0.76 and 3.22 m/year.

On the basis of the different scenarios, steady-state water distribution profiles were achieved 3.8−15.3 years after the wastewater lagoon began its operation. Given the fact that the dairy farm has been operating for the last 40 years, it is not surprising to find that the quality of the underlying groundwater has deteriorated and that increasing concentrations of chloride and nitrate, as well as traces of testosterone and estrogen, were measured in the monitoring well under the farm as compared to the upgradient groundwater (Figure 2).

Figure 4 illustrates the simulated distribution of testosterone and estrogen in the vadose zone based on two different modeling scenarios, 1 and 4 (Table 2), and distribution coefficients from selected studies (*15, 16, 26, 27*). It is clearly seen that increasing $K_{s\text{-clay}}$ and reducing $K_{d\text{-clay}}$ values resulted in deeper penetration of hormones into the sediment

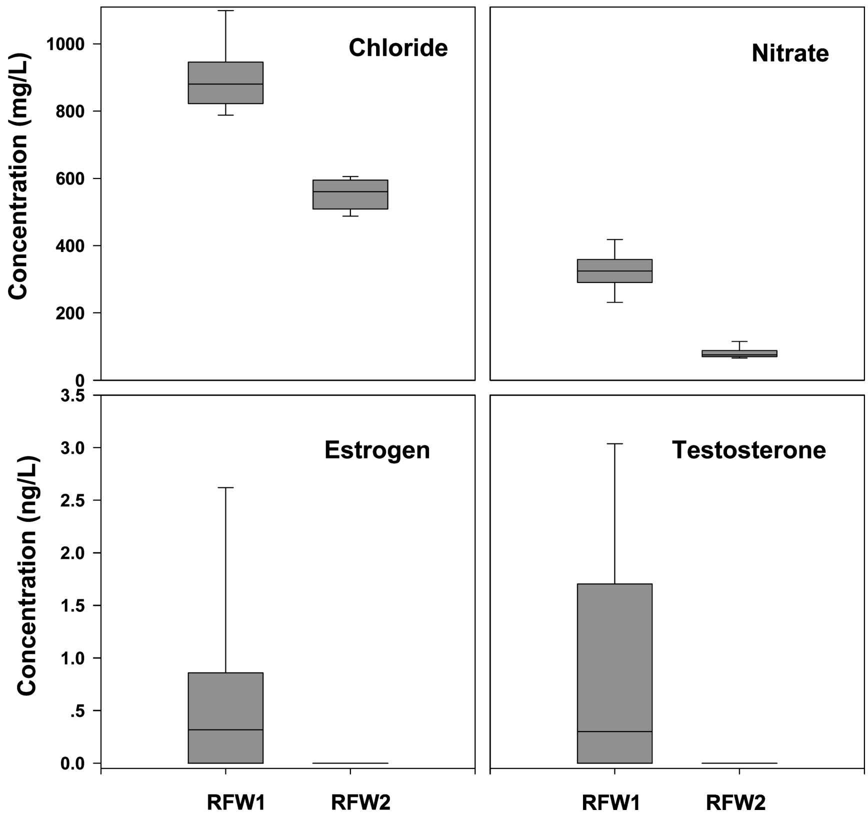

FIGURE 2. Concentrations of nitrate, chloride, testosterone, and estrogen in the monitoring wells under the dairy farm (RFW1) and under the agricultural field (RFW2). The central line in the Box-Whisker presentation indicates the median value, the upper and lower boundaries of the box indicate the 25th and 75th percentile values, and error bars indicate the 10th and 90th percentile values. Groundwater samples were taken every 4–8 weeks during 2006–2007.

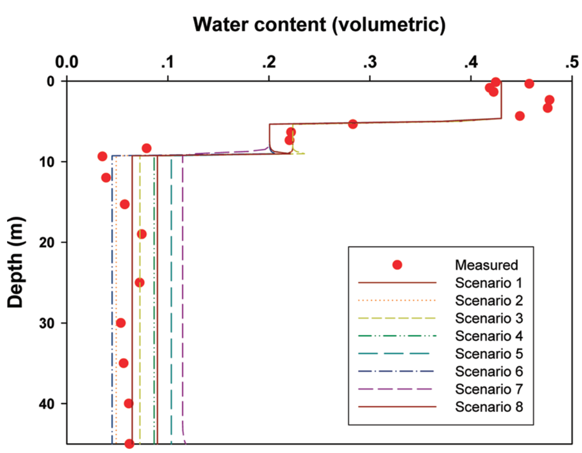

FIGURE 3. Distribution of water content in the sediment profile under the dairy-farm waste lagoon (●) and simulated profiles under different scenarios (Table 2). The water-content profiles are calculated for t = 40 years, although a steady-state in all simulations occurred 3–6 years after the wastewater lagoon began its operation.

profile. Neither of the simulated scenarios with linear distribution coefficients predicted that estrogen would leach below the clay layer underlying the wastewater lagoon (6 m). However, changing the adsorption model from linear to nonlinear (Freundlich) doubled the depth of penetration. A maximum depth of estrogen penetration after 40 years of continuous input was calculated at ~12 m (Figure 4a), with a $K_{d\text{-clay}}$ value of 10 mL/g, which is unrealistic in practice based on data concerning adsorption to clay soils. Usually, the measured $K_{d\text{-clay}}$ values are closer to 100 mL/g, which reduces the maximum depth of penetration to ~8 m. Overall, the transport of testosterone followed the same pattern as

TABLE 2. Hydraulic Parameters Used To Model Water-Content Distribution and Solute Transport

parameter	$K_{s\text{-clay}}$ (cm/day)	$Q_{r\text{-sand}}$	n_{sand}	$K_{s\text{-sand}}$ (cm/day)
scenario 1	0.17	0.065	3.6	700
scenario 2	0.17	0.02	3.6	700
scenario 3	0.17	0.02	3.6	100
scenario 4	0.08	0.065	3.6	700
scenario 5	0.08	0.065	3.6	100
scenario 6	0.08	0.02	3.6	100
scenario 7	0.08	0.02	1.6	700
scenario 8	0.08	0.02	3.6	100

that of estrogen, but with greater penetration depths due to its lower distribution coefficients (Figure 4). When simulations were conducted with a $K_{d\text{-clay}}$ value similar to that of clean sand, testosterone could reach a depth of 25 m. The low $K_{d\ sand}$ value that was assigned in this case, 0.5 mL/g, also affected the shape of the concentration profile below a depth of 8 m. This extreme scenario showed what might happen in a sandy profile if the wastewater-pond lining failed. The results in Figure 4 also illustrate a smooth decline of several orders of magnitude along the profile in the sediments, in contrast to our measurements (Figure 2), which displayed a relatively scattered and more even distribution in the sediment profile. This scattered distribution could occur because a different mechanism of transport affects hormone transport, but it could also be due to temporal changes in the hormone concentrations in the waste or due to biodegradation processes, as it has been shown that steroidal hormones can be degraded under aerobic and anaerobic conditions (*12, 14, 30*). However, we did not study the degradation of hormones in the sediments under the CAFO, and we do not know as to whether this degradation is at all

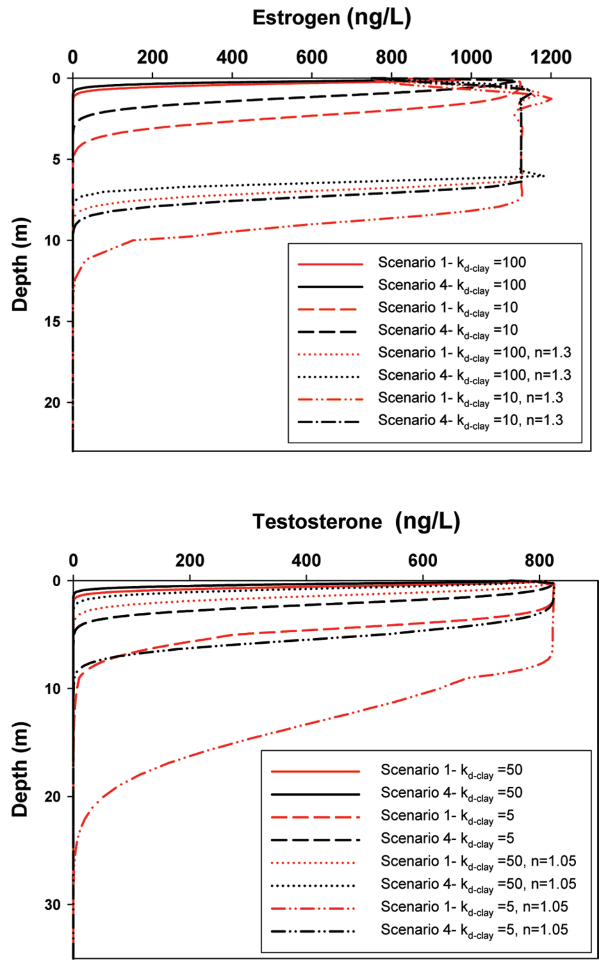

FIGURE 4. Simulated pore water concentrations of estrogen and testosterone under the dairy-farm waste lagoon. Simulations were conducted for t = 40 years with continuous variable input of hormones as described in the Materials and Methods. The values of the distribution coefficients, as well as the *n* parameter that shows deviation from linearity in the Freundlich model, appear in the legend.

significant. Nevertheless, the fact that estrogen and testosterone were found in deep sediments implies that degradation under natural conditions is limited and that we cannot rely on natural attenuation processes as an efficient site-remediation approach.

There are some limitations on the results from modeling transport processes with a 1-D model in a 3-D domain, especially with respect to lateral-dispersion processes, as discussed by Heatwole and McCray (*31*). They concluded that such lateral dispersion would tend to reduce concentrations as compared to those calculated by a 1-D model with the assumption of only longitudinal dispersion, a conservative estimate from an environmental protection perspective. In addition, lateral dispersion and unstable flow (fingering) due to discontinuous layers is unlikely to occur at our site since a gradual transition between the dominant layers was observed during drilling. Therefore, our conservative estimates due to lateral dispersion and unrealistically low adsorption coefficients clearly show that the deep penetration of hormones cannot be explained by solute transport mechanisms.

Implications for Field Monitoring and Remediation. The results presented in this study clearly show the potential seepage of hormones as well as inorganic contaminants from CAFO waste lagoons to deep groundwater, even when a relatively thick layer of clay serves as a natural barrier. We used several transport scenarios that differed mostly in their $K_{s\text{-clay}}$ and $K_{d\text{-clay}}$ values. The discrepancies between the observed and the simulated distributions of testosterone and estrogen show that solute transport with sorption alone cannot explain the distribution of hormones under the waste lagoon and suggest that other mechanisms affect hormone transport in the subsurface. We believe that further laboratory and field studies should focus on alternative transport mechanisms, such as enhanced transport due to dissolved organic carbon or colloids, both of which are found in manure (*27, 32*). Preferential flow paths also can play a significant role in contaminant transport; however, under waste lagoons, macroscale preferential flow paths (i.e., desiccation cracks) do not play a major role because the clay layer under the waste lagoon is under saturated conditions (Figure 3). We also believe that microscale preferential flow paths are likely to become clogged very quickly due to the high solids content in the manure.

Although lining with clay is still a common practice in many countries, we show here that this type of lining cannot efficiently protect the groundwater environment from waste lagoon leachates under long-term exposure. The fact that hormones were detected in different geological media and under different redox conditions suggests that their degradation in the subsurface environment is limited, and therefore, natural attenuation cannot be relied on as a removal mechanism. Although the measured concentrations of hormones in the groundwater were below the LOEL for fish and plants, it is still not clear as to how such concentrations will affect humans under long-term usage. Unless the hormones in the manure are treated, these concentrations are likely to increase in the near future and will warrant routine groundwater-resource testing for estrogenic activity to ensure public health.

Acknowledgments

This work was funded by the Division of Water Quality, Israel Water Authority. We thank Dr. Naftali Lazarovich for helpful discussions.

Supporting Information Available

Cross-section of sediments in research boreholes, detailed description of drilling and sediment-sampling procedures, and sensitivity analysis to evaluate the impact of different model input parameters on simulated water content. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Cihan, A.; Tyner, J. S.; Wright, W. C. Seal formation beneath animal waste holding ponds. *Trans. ASABE* **2006**, *49*, 1539–1544.

(2) Harter, T.; Davis, H.; Mathews, M. C.; Meyer, R. D. Shallow groundwater quality on dairy farms with irrigated forage crops. *J. Contam. Hydrol.* **2002**, *55*, 287–315.

(3) Cho, J. C.; Cho, H. B.; Kim, S. J. Heavy contamination of a subsurface aquifer and a stream by livestock wastewater in a stock farming area, Wonju, Korea. *Environ. Pollut.* **2000**, *109*, 137–146.

(4) Hooda, P. S.; Edwards, A. C.; Anderson, H. A.; Miller, A. A review of water quality concerns in livestock farming areas. *Sci. Total Environ.* **2000**, *250*, 143–167.

(5) McNab, W. W.; Singleton, M. J.; Moran, J. E.; Esser, B. K. Assessing the impact of animal waste lagoon seepage on the geochemistry of an underlying shallow aquifer. *Environ. Sci. Technol.* **2007**, *41*, 753–758.

(6) Singleton, M. J.; Esser, B. K.; Moran, J. E.; Hudson, G. B.; McNab, W. W.; Harter, T. Saturated zone denitrification: Potential for natural attenuation of nitrate contamination in shallow groundwater under dairy operations. *Environ. Sci. Technol.* **2007**, *41*, 759–765.

(7) Shore, L. S.; Gurevitz, M.; Shemesh, M. Estrogen as an environmental pollutant. *Bull. Environ. Contam. Toxicol.* **1993**, *51*, 361–366.

(8) Alon, G.; Shore, L. S.; Steinberger, Y. Correlation between levels of sex hormones progesterone, testosterone, and estrogen and ecophysiological-behavior stages in two species of desert snails (*Sphincterochila zonata* and *Sphincterochila prophetarum*) in the Northern Negev Desert. *Gen. Comp. Endrocrinol.* **2007**, *151*, 122–127.
(9) Rogan, W. J.; Ragan, N. B. Evidence of effects of environmental chemicals on the endocrine system in children. *Pediatrics* **2003**, *112*, 247–252.
(10) Routledge, E. J.; Sheahan, D.; Desbrow, C.; Brighty, G. C.; Waldock, M.; Sumpter, J. P. Identification of estrogenic chemicals in STW effluent. 2. In vivo responses in trout and roach. *Environ. Sci. Technol.* **1998**, *32*, 1559–1565.
(11) Shore, L. S.; Kapulnik, Y.; Bendor, B.; Fridman, Y.; Wininger, S.; Shemesh, M. Effects of estrone and 17-β-estradiol on vegetative growth of *Medicago sativa*. *Physiol. Plant.* **1992**, *84*, 217–222.
(12) Khanal, S. K.; Xie, B.; Thompson, M. L.; Sung, S.; Ong, S. K.; van Leeuwen, J. Fate, transport, and biodegradation of natural estrogens in the environment and engineered systems. *Environ. Sci. Technol.* **2006**, *40*, 6537–6546.
(13) Das, B. S.; Lee, L. S.; Rao, P. S. C.; Hultgren, R. P. Sorption and degradation of steroid hormones in soils during transport: Column studies and model evaluation. *Environ. Sci. Technol.* **2004**, *38*, 1460–1470.
(14) Casey, F. X. M.; Hakk, H.; Simunek, J.; Larsen, G. L. Fate and transport of testosterone in agricultural soils. *Environ. Sci. Technol.* **2004**, *38*, 790–798.
(15) Casey, F. X. M.; Simunek, J.; Lee, J.; Larsen, G. L.; Hakk, H. Sorption, mobility, and transformation of estrogenic hormones in natural soil. *J. Environ. Qual.* **2005**, *34*, 1372–1379.
(16) Lee, L. S.; Strock, T. J.; Sarmah, A. K.; Rao, P. S. C. Sorption and dissipation of testosterone, estrogens, and their primary transformation products in soils and sediment. *Environ. Sci. Technol.* **2003**, *37*, 4098–4105.
(17) Wicks, C.; Kelley, C.; Peterson, E. Estrogen in a karstic aquifer. *Ground Water* **2004**, *42*, 384–389.
(18) Swartz, C. H.; Reddy, S.; Benotti, M. J.; Yin, H. F.; Barber, L. B.; Brownawell, B. J.; Rudel, R. A. Steroid estrogens, nonylphenol ethoxylate metabolites, and other wastewater contaminants in groundwater affected by a residential septic system on Cape Cod, MA. *Environ. Sci. Technol.* **2006**, *40*, 4894–4902.
(19) Shore, L. S.; Hall, D. W.; Shemesh, M. Estrogen and Testosterone in Ground Water in the Chesapeake Bay Watershed. *Dahlia Greidinger International Symposium on Fertilization and the Environment*, Technion Israel Institute of Technology, Haifa, Israel, 1997; pp 250−255.
(20) Mansell, B. L.; Drewes, J. E. Fate of steroidal hormones during soil−aquifer treatment. *Groundwater Monit. Rem.* **2004**, *24*, 94–101.
(21) Shore, L. S.; Harelmarkowitz, E.; Gurevich, M.; Shemesh, M. Factors affecting the concentration of testosterone in poultry litter. *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.* **1993**, *28*, 1737–1749.
(22) Shore, L. S.; Shemesh, M. Naturally produced steroid hormones and their release into the environment. *Pure Appl. Chem.* **2003**, *75*, 1859–1871.
(23) Barel-Cohen, K.; Shore, L. S.; Shemesh, M.; Wenzel, A.; Mueller, J.; Kronfeld-Schor, N. Monitoring of natural and synthetic hormones in a polluted river. *J. Environ. Manage.* **2006**, *78*, 16–23.
(24) Simunek, J.; van Genuchten, M. T.; Sejna, M. *The HYDRUS-1D Software Package for Simulating the Movement of Water, Heat, and Multiple Solutes in Variably Saturated Media, version 3.0, HYDRUS Software Series 1*; Department of Environmental Sciences, University of California−Riverside: Riverside, CA, 2005; p 270
(25) Tyner, J. S.; Wright, W. C.; Lee, J. Lagoon sealing and filter cakes. *Trans. ASABE* **2006**, *49*, 527–531.
(26) Casey, F. X. M.; Larsen, G. L.; Hakk, H.; Simunek, J. Fate and transport of 17β-estradiol in soil−water systems. *Environ. Sci. Technol.* **2003**, *37*, 2400–2409.
(27) Stumpe, B.; Marschner, B. Long-term sewage sludge application and wastewater irrigation on the mineralization and sorption of 17β-estradiol and testosterone in soils. *Sci. Total Environ.* **2007**, *374*, 282–291.
(28) Peterson, E. W.; Davis, R. K.; Orndorff, H. A. 17β-estradiol as an indicator of animal waste contamination in mantled karst aquifers. *J. Environ. Qual.* **2000**, *29*, 826–834.
(29) Hanselman, T. A.; Graetz, D. A.; Wilkie, A. C. Manure-borne estrogens as potential environmental contaminants: A review. *Environ. Sci. Technol.* **2003**, *37*, 5471–5478.
(30) Colucci, M. S.; Bork, H.; Topp, E. Persistence of estrogenic hormones in agricultural soils: I. 17β-Estradiol and estrone. *J. Environ. Qual.* **2001**, *30*, 2070–2076.
(31) Heatwole, K. K.; McCray, J. E. Modeling potential vadose-zone transport of nitrogen from onsite wastewater systems at the development scale. *J. Contam. Hydrol.* **2007**, *91*, 184–201.
(32) Barkle, G. F.; Brown, T. N.; Painter, D. J. Leaching of particulate organic carbon from land-applied dairy farm effluent. *Soil Sci.* **1999**, *164*, 252–263.

ES800784M